CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-990

PHARMACOLOGY/TOXICOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-990
DATE RECEIVED BY CENTER:	2/22/06
DRUG PRODUCT:	EXFORGE® Tablets
DRUG SUBSTANCE:	Amlodipine Besylate and Valsartan
INTENDED CLINICAL POPULATION:	Hypertensive
SPONSOR:	Novartis Pharmaceuticals Corporation
REVIEW DIVISION:	Division of Cardiovascular and Renal Products
PHARM/TOX REVIEWER:	G. Jagadeesh, Ph.D.
PHARM/TOX SUPERVISOR:	Charles Resnick, Ph.D.
DIVISION DIRECTOR:	Norman Stockbridge, M.D., Ph.D.
PROJECT MANAGER:	Nguyen Quynh

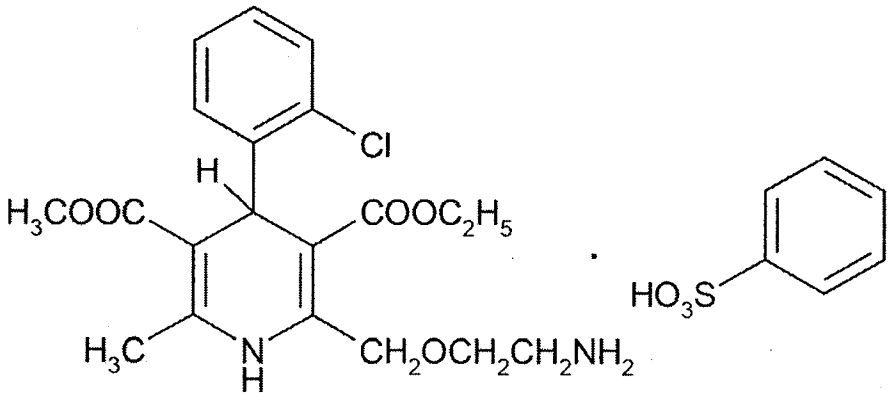
Date of review submission to Division File System (DFS): November 07, 2006

NDA number: 21,990
Date of Submission: 2-22-06
Center Receipt Date: 2-22-06
Sponsor: Novartis Pharmaceuticals Corporation
Manufacturer of Drug Substance: _____ Valsartan is from Novartis Pharmaceuticals Corporation.
Manufacturer of Drug Product: Novartis Pharmaceuticals Corporation
Reviewer: G. Jagadeesh, Ph.D.
Division: Division of Cardiovascular and Renal products
Review completion date: 11-07-2006

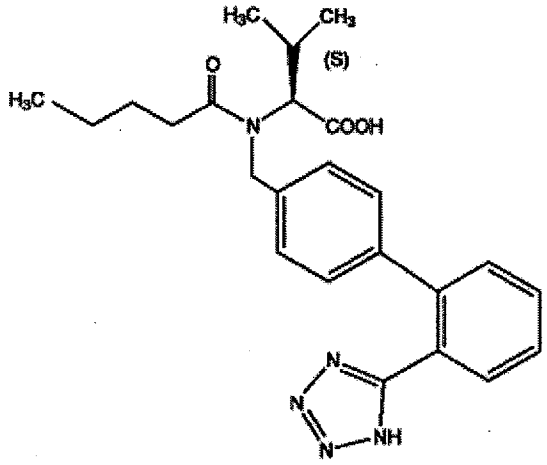
Drug Product: EXFORGE®

Drug Substances

Generic name: **Amlodipine Besylate**
Code name: LBT873-DMA.002
Chemical name: (*RS*)-2-[(2'-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl ester, 5-methyl ester, benzene sulfonate.
Chemistry: Amlodipine is a racemic mixture (R and S isomers). It is a white to pale yellow crystalline powder slightly soluble in water and sparingly soluble in ethanol.
CAS registry number: 1114790-99-6 (besylate salt form)
88150-42-9 (free base form)
Molecular formula/molecular weight: $C_{20}H_{25}ClN_2O_5 \cdot C_6H_5SO_3H$ / 567.06 (besylate)



Generic name: **Valsartan**
Code name: CGP 48933
Chemical name: (*S*)- N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-L-valine.
Chemistry: Valsartan contains two acidic functions and includes one asymmetric center. It is free diacid, hydrophilic and the pure *S*-enantiomer. The corresponding (R)-enantiomer is less active in biological tests. It is a white, microcrystalline and soluble in water.



CAS registry number: 173334-58-2
Molecular formula/molecular weight: $C_{24}H_{29}N_5O_3$/ 435.5 (free base)

Related Applications: Clinical trials supporting the current NDA were conducted under Novartis' IND 65,174. Novartis' NDA 20,665 for Valsartan (Diovan®) was approved for the treatment of hypertension in 1996. Pfizer's NDA 19,787 for racemic amlodipine besylate (Norvasc®) was approved for the treatment of hypertension, chronic stable angina and vasospastic angina in 1992.

Drug Class: Valsartan: Angiotensin II receptor class 1 (AT_1 receptor) antagonist
Amlodipine: Dihydropyridine calcium channel blocker

Intended Clinical Population: Hypertensive subjects

Clinical Formulation: The tablets are formulated in ——— with amlodipine besylate equivalent to 5 mg or 10 mg of amlodipine free-base combined with 80, 160 or 320 mg of valsartan. The following table lists proposed final commercial formulations ——— strength is not included in the labeling).

COMPOSITION OF AMLODIPINE BESYLATE AND VALSARTAN (VAA489) FILM-COATED TABLET

Ingredient	5/160 mg 6001522.001	10/160 mg 6001267.003	5/320 mg 6001265.007	10/320 mg 6001735.004
Component (mg)				
Valsartan				
Amlodipine besylate				
Microcrystalline cellulose				
Crospovidone				
Colloidal silicon dioxide				
Magnesium stearate				
Sodium starch glycolate				
Iron oxide yellow				

[2] corresponds to 5 mg of Amlodipine free base
[3] corresponds to 10 mg of Amlodipine free base
[4] removed during processing

The film coating contains hypromellose, iron oxides, polyethylene glycol, talc and titanium dioxide.

Route of Administration: Oral

Proposed Dosage Regimen: One tablet daily.

Disclaimer: Unless indicated otherwise, tables and graphs (with or without editorial corrections by the reviewer) are taken from the sponsor's submission.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Background

The rationale for combining two antihypertensive agents from different pharmacologic classes is based on the expectation that the combination will exert an additive or synergistic antihypertensive effect when compared to single drug treatment. Such combinations permit simultaneous targeting of multiple physiological systems involved in the regulation of blood pressure. In addition, the pharmacological action of the two agents combined may improve patient compliance and enhance tolerability by reducing the incidence of certain side effects that are more prevalent when the drugs are used alone. The sixth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure notes that "combinations of low doses of two agents from different classes have been shown to provide additional antihypertensive efficacy, thereby minimizing the likelihood of dose-dependent adverse effects" (*Arch Inten Med.*, 157: 2413-2446, 1997).

In the 1960s, the combination of reserpine with HCTZ and hydralazine became the first fixed-dose combination therapy approved for use. This was followed by thiazide/potassium-sparing diuretic, ß-adrenergic blocker/thiazide diuretic and clonidine/thiazide diuretic combinations in the 1970s, ACE inhibitor/thiazide diuretic combinations in 1980s, and low dose ß-blocker/thiazide diuretic and ACE inhibitor or AT-1 receptor antagonist/calcium channel antagonist combinations in the 1990s. The latter combination has been shown to be more effective in lowering blood pressure, with fewer undesirable side effects, than either agent alone. Reduction of circulating angiotensin II levels and calcium channel blockade have additive effects in lowering blood pressure. VAA489 (Exforge®) is a new combination tablet containing the AT-1 receptor antagonist, valsartan, and the calcium channel blocker, amlodipine besylate, for the treatment of hypertension. Both valsartan and amlodipine besylate are currently marketed for the treatment of hypertension.

II. Recommendations

A. **Recommendation on Approvability:** Approvable

B. **Recommendations for Additional Nonclinical Studies:** None

C. **Recommendations for Labeling:** Those sections of the proposed labeling (EDR version dated August 15, 2006) that deal with nonclinical studies covered by this review are considered satisfactory with the following exceptions.

1. Under PRECAUTIONS,
   Carcinogenesis/Mutagenesis/Impairment of Fertility,
   the sponsor's proposed text reads as follows:

"Rats and mice treated with amlodipine maleate in the diet for up to two years, at concentrations calculated to provide daily dosage levels of 0.5, 1.25, and 2.5 mg/kg/day amlodipine showed no

evidence of a carcinogenic effect of the drug. For the mouse, the highest dose was on mg/m^2 basis, similar to the maximum recommended human dose of 10 mg/day amlodipine*. For the rat, the highest dose was, on a mg/m^2 basis, about twice the maximum recommended human dose*.

Mutagenicity studies conducted with amlodipine maleate revealed no drug related effects at either the gene or chromosomal levels. There was no effect on the fertility of rats treated orally with amlodipine (males for 64 days and females for 14 days prior to mating) at doses up to 10 mg/kg/day amlodipine (8 times* the maximum recommended human dose of 10 mg/kg/day on a mg/m^2 basis).

*Based on patient weight of 50 kg.

There was no evidence of carcinogenicity when valsartan was administered in the diet to mice and rats for up to 2 years at doses up to 160 and 200 mg/kg/day, respectively. These doses in mice and rats are about— and 6 times, respectively, the maximum recommended human dose on a mg/m^2 basis. (Calculations assume an oral dose of 320 mg/day and a 60-kg patient.) Mutagenicity assays did not reveal any valsartan-related effects at either the gene or chromosome level. These assays included bacterial mutagenicity tests with Salmonella ——— and E coli; a gene mutation test with Chinese hamster V79 cells; a cytogenetic test with Chinese hamster ovary cells; and a rat micronucleus test.

Valsartan had no adverse effects on the reproductive performance of male or female rats at oral doses up to 200 mg/kg/day. This dose is 6 times the maximum recommended human dose on a mg/m2 basis. ———————————————————

The above text for amlodipine and valsartan, the drug components of Exforge®, is taken from the approved labeling for Norvasc® and Diovan®. The rodent:human dose ratios in both cases are based on body surface area, with an assumed patient weight of 50 kg for amlodipine and 60 kg for valsartan. These multiples are recalculated by this reviewer using a similar human body weight, 60 kg, for both drugs. The following statement incorporates our recommended changes (underlined):

"Rats and mice treated with amlodipine maleate in the diet for up to two years, at concentrations calculated to provide daily dosage levels of 0.5, 1.25, and 2.5 mg amlodipine/kg/day, showed no evidence of a carcinogenic effect of the drug. For the mouse, the highest dose was, on mg/m^2 basis, similar to the maximum recommended human dose (MRHD) of 10 mg amlodipine/day. For the rat, the highest dose was, on a mg/m^2 basis, about two and a half times the maximum recommended human dose. (Calculations based on a 60 kg patient.)

Mutagenicity studies conducted with amlodipine maleate revealed no drug-related effects at either the gene or chromosome level.

There was no effect on the fertility of rats treated orally with amlodipine maleate (males for 64 days and females for 14 days prior to mating) at doses of up to 10 mg amlodipine/kg/day (about 10 times the MRHD of 10 mg/day on a mg/m^2 basis).

There was no evidence of carcinogenicity when valsartan was administered in the diet to mice and rats for up to 2 years at concentrations calculated to provide doses of up to 160 and 200 mg/kg/day, respectively. These doses in mice and rats are about 2.4 and 6 times, respectively, the maximum recommended human dose of 320 mg/day on a mg/m^2 basis. (Calculations based on a 60 kg patient.)

Mutagenicity assays did not reveal any valsartan-related effects at either the gene or chromosome level. These assays included bacterial mutagenicity tests with Salmonella and E. coli, a gene mutation test with Chinese hamster V79 cells, a cytogenetic test with Chinese hamster ovary cells, and a rat micronucleus test.

Valsartan had no adverse effects on the reproductive performance of male or female rats at oral doses of up to 200 mg/kg/day. This dose is about 6 times the maximum recommended human dose on a mg/m^2 basis."

2. Under **Pregnancy,**
   Teratogenic Effects,
   the sponsor's proposed text summarizing the results of studies in rats and rabbits reads as follows:

"No evidence of teratogenicity or other embryo/fetal toxicity was found when pregnant rats and rabbits were treated orally with amlodipine maleate at doses up to 10 mg/kg/day amlodipine ——— during their respective periods of major organogenesis. However, litter size was significantly decreased (by about 50%) and the number of intrauterine deaths was significantly increased (about 5-fold) in rats receiving amlodipine maleate at a dose equivalent to 10 mg/kg/day amlodipine for 14 days before mating and throughout mating and gestation. Amlodipine maleate has been shown to prolong both the gestation period and the duration of labor in rats at this dose. There are no adequate and well-controlled studies in pregnant women. Amlodipine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

*Based on patient weight of 50 kg.

No teratogenic effects were observed when valsartan was administered to pregnant mice and rats at oral doses up to 600 mg/kg/day and to pregnant rabbits at oral doses up to 10 mg/kg/day. However, significant decreases in fetal weight, pup birth weight, pup survival rate and slight delays in developmental milestones were observed in studies in which parental rats were treated with valsartan at oral, maternally toxic (reduction in body weight gain and food consumption) doses of 600 mg/kg/day during organogenesis or late gestation and lactation. In rabbits, fetotoxicity (i.e., resorptions, litter loss, abortions, and low body weight) associated with maternal toxicity (mortality) was observed at doses of 5 and 10 mg/kg/day. The no observed adverse effect doses of 600, 200 and 2 mg/kg/day in mice, rat and rabbits represent 9, 6, and 0.1 times ——— the maximum recommended human dose ——— (Calculations ——— 60-kg ———

In the oral embryo-fetal development study in rats using ——— at doses equivalent to 5 mg/kg/day amlodipine plus 80 mg/kg/day valsartan, 10 mg/kg/day amlodipine plus 160 mg/kg/day valsartan, and 20 mg/kg/day amlodipine plus 320 mg/kg/day valsartan, treatment-related maternal and fetal effects (developmental delays and alterations noted in the presence of significant maternal toxicity) were noted at the high dose combination ——— no-observed-adverse-effect level (NOAEL) was 5 mg/kg/day amlodipine plus 80 mg/kg/day valsartan while the embryo-fetal ———10 mg/kg/day amlodipine plus 160 mg/kg/day valsartan."

The above text for amlodipine and valsartan, the drug components of Exforge®, is taken from the approved labeling for Norvasc® and Diovan®. The rodent:human dose ratios in both cases are based on body surface area, with an assumed patient weight of 50 kg for amlodipine and 60 kg for valsartan. These multiples are recalculated by this reviewer using a similar human body weight, 60 kg, for both drugs. For the valsartan and amlodipine combination study, rat-to-human mg/m^2 dosage multiples or systemic exposure multiples are not provided. The following statement incorporates our recommended changes (underlined):

"No evidence of teratogenicity or other embryo/fetal toxicity was found when pregnant rats and rabbits were treated orally with amlodipine maleate at doses of up to 10 mg amlodipine/kg/day (respectively, about 10 and 20 times the maximum recommended human dose [MRHD] of 10 mg amlodipine on a mg/m^2 basis) during their respective periods of major organogenesis. (Calculations based on a patient weight of 60 kg.) However, litter size was significantly decreased (by about 50%) and the number of intrauterine deaths was significantly increased (about 5-fold) for rats receiving amlodipine maleate at a dose equivalent to 10 mg amlodipine/kg/day for 14 days before mating and throughout mating and gestation. Amlodipine maleate has been shown to prolong both the gestation period and the duration of labor in rats at this dose. There are no adequate and well-controlled studies in pregnant women. Amlodipine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

No teratogenic effects were observed when valsartan was administered to pregnant mice and rats at oral doses of up to 600 mg/kg/day and to pregnant rabbits at oral doses of up to 10 mg/kg/day. However, significant decreases in fetal weight, pup birth weight, pup survival rate, and slight delays in developmental milestones were observed in studies in which parental rats were treated with valsartan at oral, maternally toxic (reduction in body weight gain and food consumption) doses of 600 mg/kg/day during organogenesis or late gestation and lactation. In rabbits, fetotoxicity (i.e., resorptions, litter loss, abortions, and low body weight) associated with maternal toxicity (mortality) was observed at doses of 5 and 10 mg/kg/day. The no observed adverse effect doses of 600, 200 and 2 mg/kg/day in mice, rats and rabbits, respectively, are about 9, 6 and 0.1 times the MRHD of 320 mg/day on a mg/m^2 basis. (Calculations based on a patient weight of 60 kg.)

In the oral embryo-fetal development study in rats using amlodipine besylate plus valsartan at doses equivalent to 5 mg/kg/day amlodipine plus 80 mg/kg/day valsartan, 10 mg/kg/day amlodipine plus 160 mg/kg/day valsartan, and 20 mg/kg/day amlodipine plus 320 mg/kg/day

valsartan, treatment-related maternal and fetal effects (developmental delays and alterations noted in the presence of significant maternal toxicity) were noted with the high dose combination. The no-observed-adverse-effect level (NOAEL) for embryo-fetal effects was 10 mg/kg/day amlodipine plus 160 mg/kg/day valsartan. On a systemic exposure [$AUC_{0-\infty}$] basis, these doses are, respectively, 4.3 and 2.7 times the systemic exposure [$AUC_{0-\infty}$] in humans receiving the MRHD (320:10 mg/60 kg)."

III. Summary of Nonclinical Findings

The sponsor has not performed pharmacology and ADME studies for the combination product.

A. **Brief Overview of Toxicology:** To support the chronic administration of the valsartan besylate/amlodipine combination to adult hypertensive patients, repeat dose toxicity studies (up to 13 weeks) were performed in rats and marmosets. Developmental toxicity was evaluated in pregnant rats. In all of these studies, valsartan and amlodipine were administered orally, by gavage, at a ratio of 16:1. (All doses and dose ratios in this review are presented in terms of the amlodipine base.)

Repeat dose toxicity studies of the valsartan/amlodipine combination in rats and marmosets revealed adverse effects on the gastrointestinal tract (GIT), kidney, erythroid parameters and heart. Each of these effects could be attributed to known effects of one or both components (valsartan and/or amlodipine) and, in most cases, to exaggerated pharmacological effects. Histopathological lesions indicative of GIT irritation were observed in the glandular stomach in rats and were characterized by inflammation, ulcers/erosions at doses as low as 48:3 mg (valsartan:amlodipine)/kg/day in males and 120:7.5 mg/kg/day in females. Males were more affected than females. This effect was not noted in recovery group animals. Stomach inflammation with the test substance was absent in marmosets. On the other hand, moderate to marked erosive or ulcerative inflammation of the large intestine (cecum and/or colon) was evident in marmosets treated with amlodipine, alone or in combination with valsartan [160:10→80:5 mg (valsartan:amlodipine)/kg/day]. The effect was also noted in recovery group animals. The highest dose of the combination resulted in mortality for marmosets (at 160:10 mg/kg/day) and rats (at 240:15 mg/kg/day) due to erosive/ulcerative inflammation of the gut. GIT lesions have previously been associated with amlodipine and/or valsartan administration.

An increase in the incidence and/or severity of nephropathy (characterized by tubular basophilia, dilatation and casts, and interstitial lymphocytic inflammation) was noted for both sexes of rats (≥120:7.5 mg/kg/day) and marmosets (160:10→80:5 mg/kg/day) treated with valsartan, alone or in combination with amlodipine. In addition, medial hypertrophy of renal cortical arterioles and juxtaglomerular cells, coupled with an increase in BUN values, was noted for both species at the above doses. These findings were absent in recovery group marmosets but not in recovery group rats. The sponsor attributes the renal effects to an exaggerated pharmacological

effect of valsartan: decreased renal perfusion and subsequent ischemia following a prolonged hypotensive effect.
Valsartan-attributed significant decreases in erythroid parameters (erythrocytes, hemoglobin, hematocrit), reticulocyte counts and/or erythropoiesis in the bone marrow and spleen were noted for both sexes of rats [at doses ≥120:7.5 mg (valsartan:amlodipine)/kg/day and 240 mg valsartan/kg/day] and marmosets [at doses of 160:10→80:5 mg (valsartan:amlodipine)/kg/day and 160→80 mg valsartan/kg/day. These effects are similar to those exhibited by other angiotensin receptor blockers and ACE inhibitors and are attributed to the opposing effect of valsartan on angiotensin II stimulated erythropoiesis.
Nondose-dependent decreases ($p < 0.05$) in absolute and relative heart weights were noted in rats but not marmosets at doses as low as 48:3 mg (valsartan:amlodipine)/kg/day and 240 mg valsartan/kg/day with no accompanying histopathological findings. This was considered secondary to the expected hypotensive effects of the drug(s), resulting in decreased cardiac overload. In marmosets, lymphocytic inflammation, hemorrhage and/or edema were noted in the atrial myocardium for both sexes treated with amlodipine alone (10→5 mg/kg/day) or in combination with valsartan (160:10→80:5 mg/kg/day). Similar findings have been previously reported with amlodipine alone.
Additional findings in rats included a nondose-dependent reduction in mean body weight relative to concurrent control (5.5 to 16%) at doses as low as 48:3 mg/kg/day. Though body weights had recovered somewhat by the end of the recovery period, they were still below control weights ($p < 0.05$ for 240:15 mg/kg/day group). For marmosets, body weight loss relative to control or baseline was dose-dependent in terms of the numbers of animals affected, the onset and the severity of the loss. Doses as low as 40:2.5 mg/kg/day resulted in weight loss of up to 6-11% over the course of the study. A dose of 160:10 mg/kg/day could not be tolerated, resulting in severe and rapid body weight loss and two animals died on study day 8. Dosing for this group was suspended between study days 10 and 14 and resumed on day 15 with a reduction in dose from 160:10 to 80:5 mg (valsartan: amlodipine)/kg/day.

Reproductive Toxicity: The teratogenic potential of a valsartan/amlodipine combination was evaluated in an embryo-development study in rats. No evidence of teratogenicity was noted at doses of up to 320:20 mg/kg/day. However, at this dose, decreased birth weight, increased incidence of dilated ureters and skeletal findings of misshapen sternebrae and un-ossified forepaw phalanges were noted which may have been related to maternal toxicity (significant decreases in body weight gain and food consumption relative to control) noted at 160:10 or more mg/kg/day and at 20 mg amlodipine/kg/day. Dilated ureters were also noted at 320 mg valsartan/kg/day. The NOAEL for embryo-fetal effects was 160:10 mg (valsartan:amlodipine)/kg/day. These doses were associated with systemic exposures (AUCs) which were, respectively, 2.7 and 4.3 times the systemic exposure (AUC) in humans receiving the MRHD (320:10 mg/60 kg).

B. **Nonclinical Safety Issues Relevant to Clinical Use**

In toxicology studies with rats and marmosets, a 16:1 valsartan:amlodipine combination was associated with findings in the kidney, gastrointestinal tract, erythrocyte parameters and heart. Each of these effects could be attributed to known effects of valsartan and/or amlodipine. Most of the above effects, except for erosive/ulcerative lesions in the gastrointestinal tract, were associated with exaggerated pharmacological effects of valsartan and/or amlodipine. Deaths of rats and marmosets receiving the highest combination dose were attributed to gastric lesions. The combined administration of valsartan and amlodipine besylate to rats and marmosets had greater adverse effects than treatment with valsartan or amlodipine besylate alone. Though the combination was not teratogenic in rats, there was an indication of developmental delays in the presence of significant maternal toxicity at the highest dose tested.

Systemic exposures to valsartan and amlodipine (AUCs) in rats and marmosets treated with the combination were compared to systemic exposures in humans treated with 320 mg valsartan and 10 mg amlodipine. Exposures to valsartan and amlodipine at NOAELs in rats (not resulting in GI erosions/ulcers or renal lesions) were only 0.6 to 2.0 times, respectively (based on AUC values), exposures in humans at 320:10 mg (valsartan:amlodipine)/day, the maximum recommended human dose (MRHD). The NOAEL exposures in marmosets were less than a third of the valsartan or amlodipine exposures in human at the MRHD, indicating the absence of a safety margin for humans. In spite of these concerns, the combination product can still be used safely in humans for the treatment of hypertension because the target organ toxicities are monitorable and attributable to the individual drugs of the combination which are currently approved for use in this patient population and have often been used concomitantly.

IV. Administrative

Reviewer's Signature

Supervisor Signature: Concurrence

PHARMACOLOGY/TOXICOLOGY REVIEW

1.0. PHARMACODYNAMICS: NO STUDIES CONDUCTED

2.0. DRUG DISPOSITION: NO STUDIES CONDUCTED

3.0. TOXICOLOGY

3.1. Repeat Dose Toxicity

3.1.1. 13 Week Oral Gavage Study in S-D Rats With a 4 Week Recovery

Key Study Findings: Gavage administration of valsartan and amlodipine at the high combination dose (240:15 mg/kg/day) resulted in sacrifice of a male and a female in moribund condition on days 38 and 24, respectively. Gastric irritation (ulceration/erosion) was noted in both sexes at doses of 120 or more mg valsartan/kg/day, either alone or in combination with amlodipine (effect also noted in recovery group animals). Inflammation of the stomach was observed in males at 48:3 or more mg/kg/day. Kidney findings representing the early stages of chronic progressive nephropathy (also in recovery group animals) along with moderate increases in BUN and creatinine were noted in all drug-trated groups. Additional findings included nondose-dependent decreases in mean body weight relative to concurrent control at all dose levels. Decreases in erythroid parameters relative to control were noted for both sexes in mid and high dose combination and valsartan alone groups. A NOAEL was not reached in this study.

Study No.: 0470164
Location of Report: EDR
Conducting Laboratory and Location: ———————————

Dates of Study: The animals were dosed on October 13, 2004 and necropsied between January 13 and February 14, 2005
GLP Compliance: Yes
QA'd Report: yes (X) no ( )
Drug, Lot #: Amlodipine besylate, batch #TY003F03, ——— Valsartan, lot #1030765000, ——— pure
Formulation: The drugs were suspended in 0.5% (w/v) Klucel aqueous solution in weeks 1, 7 and 13.
Animals

Species/Strain: Rats, Sprague Dawley (Crl:CD(SD)IGSBR, from Charles River)
#/Sex/Group: 10/sex/group. An additional 6 animals/sex/group were included for the control, high dose combination, amlodipine and valsartan alone groups to serve as recovery animals for 4 week recovery period.
Age: 8 weeks old at initiation of dosing
Weight: Males: 248.4-300.8 gm, Females: 168.8-216.4 gm, at initiation of dosing

Husbandry: Animals were housed in same sex pairs, except during the urine collection period. Food and water were available *ad libitum* except for study defined fasting procedures and during the urine collection period.

Dosing

Doses: Valsartan and amlodipine were administered (at a ratio of 16:1) at doses of 0:0, 48:3, 120:7.5 or 240:15 mg/kg/day. Two additional groups of rats received either valsartan or amlodipine at 240 or 15 mg/kg/day, respectively (Table 3.1.1.1). The doses were selected on the basis of a 2 week oral study in S-D rats in which gavage administration of valsartan and amlodipine at doses of 80:5 or more mg/kg/day resulted in decreases in mean body weight and/or mean body weight gain in both sexes that ranged from 21 to 66%, relative to control. (Further details as to quantitation of this effect not provided.) A significant decrease in mean body weight gain was also noted in animals treated with either drug alone (valsartan at 480 or amlodipine at 30 mg/kg/day). There were also decreases in erythroid parameters and histopathological changes in the stomach (gastric erosion and related changes), decreased extramedullary hematopoiesis in the spleen and decreased hematopoietic tissue in the bone marrow in combination groups at doses of 80:5 or more mg/kg/day and in the valsartan alone (480 mg/kg/day) group.

TABLE 3.1.1.1
STUDY DESIGN

Group	Number/sex	Animal numbers		Dose* (mg/kg/day) Valsartan/ Amlodipine base (salt)**	Concentration (mg/mL) Valsartan/ Amlodipine Salt**
		males	females		
1 Control	10 +6 recovery	1001-10 1011-16	1501-10 1511-16	0	0
2 Low Combination	10	2001-10	2501-10	48/3(4.2)	4.8/0.42
3 Mid Combination	10	3001-10	3501-10	120/7.5 (10.4)	12/1.04
4 High Combination	10 + 6 recovery	4001-10 4011-16	4501-10 4511-16	240/15 (20.8)	24/2.08
5 High (valsartan)	10 + 6 recovery	5001-10 5011-16	5501-10 5511-16	240	24
6 High (amlodipine)	10 + 6 recovery	6001-10 6011-16	6501-10 6511-16	15 (20.8)	2.08

*Doses were not corrected for active moiety.

**Salt/base ratio for amlodipine besylate is 1.387.

Route, Mode and Duration of Administration: Orally by gavage (10 ml/kg), once daily, for 13 weeks. Recovery phase animals were treated for the same duration but were killed 4 weeks later. Control animals received the vehicle.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for clinical signs and mortality.
Body Weight and Food Consumption: Recorded once before treatment and once weekly during the dosing and recovery periods.
Ophthalmology: Conducted once pretest and on all surviving control and high dose animals (groups 1, 4, 6 and 6) during week 12.
Hematology and Clinical Biochemistry: Blood samples were collected from all surviving animals in weeks 5, 13 and recovery week 4 for hematology (erythrocytes, hematocrit, hemoglobin, Wintrobe indices, red cell distribution width, reticulocytes, white blood cell count, white blood cell differential, platelets) and blood chemistry (ALT, AST, AP, total bilirubin, total protein, albumin, globulins, glucose, urea, creatinine, creatine kinase, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, cholesterol, A/G ratio) examinations. Blood was drawn from the retro-orbital plexus under light isoflurane anesthesia.
Urinalysis: Urine samples were collected from individual animals for up to 5 hr and analyzed for specific gravity, bilirubin, blood, glucose, ketones, protein, urobilinogen, pH). Food and water were removed during collection.
Pathology: Animals were fasted overnight prior to terminal necropsy and weighed before sacrifice. A complete necropsy was conducted on all animals with a recording of macroscopic observations for all tissues listed in Table 3.1.1.2. At scheduled sacrifices, blood samples were collected for genomics from all surviving animals from vena cava and abdominal aorta. Organ weights were recorded only at scheduled sacrifices. Immediately after weighing, samples from the liver, kidney, spleen, heart (apex) and mesenteric lymph nodes were frozen for possible investigational gene expression analysis[1]. Microscopic examinations were performed on all tissues listed in Table 3.1.1.2 from all animals in the control and high dose non-recovery groups (groups 1, 4, 5 and 6) and for all unscheduled deaths/sacrifices. All gross lesions and the following organs were processed for microscopic evaluation from all animals including recovery groups: kidneys, spleen, stomach, small and large intestines, adrenal, thymus, bone (femur and tibia), bone marrow, mammary gland, salivary gland and lacrimal gland.
Toxicokinetics: Blood samples were collected from the retro-orbital plexus of the non-recovery animals under light isoflurane anesthesia on day 1 and in week 10 at 0.5, 1, 2, 6 and 24 hr after dosing (2 rats/sex/group/time point).

[1] Gene expression analyses were not considered a part of this study and thus were not included in this report.

TABLE 3.1.1.2

Tissue list for collection, weighing (W) and/or processing (P)

W	P	adrenal	W	P	ovary
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
W	P	brain	W	P	pituitary
	P	cecum	W	P	prostate
	P	cervix		P	rectum
	P	colon		P	salivary gland
	P	duodenum		P	sciatic nerve
	P	epididymis		P	seminal vesicle
	P	esophagus		P	skeletal muscle
	P	eye		P	skin
	P	femur/tibia		P	spinal cord
	P	harderian gland	W	P	spleen
W	P	heart		P	sternum
	P	ileum		P	stomach
	P	jejunum	W	P	testis
W	P	kidney	W	P	thymus
	P	lacrimal gland	W	P	thyroid
W	P	liver		P	tongue
	P	lung		P	trachea
	P	lymph node – bronchial		P	urinary bladder
	P	lymph node – mandibular	W	P	uterus
	P	lymph node – mesenteric		P	vagina
	P	mammary gland		P	macroscopic lesions
		nasal passage			animal identification

Results

Analysis of Formulations: The formulation was stable for at least 12 days at 6°C and for at least 4 hr at room temperature. Mean concentrations of all samples analyzed were in the range of 94% to 112% of target concentrations.

Mortality: Fourteen animals died or were sacrificed during the study. Of these, only two, a male (#4011) and a female (#4505) receiving 249:15 mg (valsartan:amlodipine)/kg/day sacrificed in moribund condition on days 38 and 24, respectively, were considered to have succumbed to an effect of the test substance. The male had gastritis accompanied by marked ulceration of the glandular stomach, while the female manifested multiple gastric erosions of moderate severity. The remaining 12 animals, including a male in the control group, died because of gavage errors, as evidenced by perforation or hemorrhagic fluid contents in the stomach.

TABLE 3.1.1.3
FOUND DEAD OR EUTHANIZED *IN EXTREMIS*

VAL:AML mg/kg/day	# of deaths	Animal #, Mortality on study day
Control	1 M	#1002 euthanized *in extremis* on day 63[π]
48:3	1 M	#2010 found dead, post dose on day 13[π]
	1 F	#2502 euthanized *in extremis* on day 73[π]
120:7.5	2 F	#3504 found dead, post dose on day 64
240:15	1 M	#4011 (recovery) humane sacrifice on day 38[§]
	3 F	#4504 #4505 euthanized in moribund condition on day 24[§] #4516 (recovery) found dead on day 49[*]
0:15	2 M	#6001 found dead on day 11 #6006 found dead on day 40
	3 F	#6504 on day 11 #6505 on day 64 #6506 euthanized *in extremis* on day 32

[π]: Cause of death was suspected misgavage
[§]: Treatment-related death, histopathological findings in stomach (ulcers and/erosions)

Clinical Signs: Test substance-related clinical signs included reddened skin of males in all dose groups with high incidences in the high dose combination group and the two mono drug groups, and in females at 15 mg amlodipine/kg/day. These signs were also noted at the end of recovery. Clinical signs noted in the moribund animals were pale appearance, thin, dehydration, abdominal distention, hunched posture and decreased locomotor activities.

Body Weights: A nondose-dependent decreases in mean body weight relative to concurrent control was noted for males (7.2, 11.9*. 15.8*, 14.3* and 13.8%*) and females (6.1, 6.5, 7.3*, 5.5 and 12.3%*, (*=p <0.05)) at 48:3, 120:7.5, 240:15, 240:0 or 0:15 (valsartan: amlodipine) mg/kg/day, respectively. Mean body weights at the end of recovery (day 29) for both sexes were still 3 to 13% below that of the control group (p <0.05 for high dose combination male group, which was 13% below control).

Food Consumption: On study day 92, a significant decrease in mean food consumption relative to control was noted for males (5.4, 4.3. 11.1*, 12.5* and 8.6% (*=p <0.05)) and females (6.1, 4.1, 4.1, 5.6 and 2.%) at 48:3, 120:7.5, 240:15, 240:0 or 0:15 (valsartan: amlodipine) mg/kg/day, respectively . The decreases in food consumption correlated with the decreases in mean body weights and body weight gains. Mean food consumption during the recovery period for both sexes was comparable to consumption of control animals.

Ophthalmoscopy: No remarkable ocular changes

Hematology: Decreases in erythroid parameters relative to control were noted in both sexes in mid and high dose combination and valsartan alone groups (Table 3.1.1.3). On the other hand, a small increase in these parameters was noted for both sexes receiving amlodipine alone. The erythroid parameters of recovery group animals were comparable to the concurrent control.

TABLE 3.1.1.3
NOTEWORTHY FINDINGS FOR HEMATOLOGY PARAMETERS

Parameter	Dose (mg/kg/day)											
	Control		48:3		120:7.5		240:15		240:0		0:15	
	M	F	M	F	M	F	M	F	M	F	M	F
RBC count (M/μL) d34	8.54	7.98	-	-	7.78	7.19	7.65	7.11	7.60	7.23	9.14	8.85
RBC count (M/μL) d90	9.17	8.15	-	-	8.59	7.65	8.40	7.74	8.38	7.77	9.67	9.04
Hemoglobin conc. (g/dL) d34	16.4	15.8	-	-	15.1	14.0	14.7	13.7	14.7	14.0	17.0	16.6
Hemoglobin conc. (g/dL) d90	16.6	15.7	-	-	--	14.6	15.7	14.7	15.7	14.9	-	16.6
Hematocrit (%) d34	47.4	44.1	-	-	43.2	39.1	41.9	38.2	42.1	39.6	49.8	47.1
Hematocrit (%) d90	48.0	44.3	-	-		41.6	44.8	41.5	45.3	42.2	-	46.5
Mean red cell volume (fL) d34	-	55.3	-	-	--	-	-	-	-	-	-	53.3
Mean red cell volume (fL) d90	-	54.4	-	-	--	-	-	-	-	-	-	51.5
Abslt reticulocytes (K/μL) d34	-	178.3	-	-	-	-	-	-	-	-	-	229.7
Abslt reticulocytes (K/μL) d90	187.2	158.1	-	-	145.8	-	155.8	-	136.6	-	-	235.1

No entries (-) are made in low dose combination group because of no noteworthy findings.

Clinical Chemistry: Mild to moderate increases in blood urea nitrogen (up to 4-fold) and creatinine (up to 1.6-fold) were noted in animals of both sexes treated with the valsartan: amlodipine combination at 120:7.5 or more mg/kg/day or valsartan alone at 120:0 mg/kg/day on days 34 and/or 90. Males were more affected than females. Other changes noted were a mild increase in serum potassium and phosphorus affecting one or both sexes in mid and high dose combination and valsartan alone groups (Table 3.1.1.4). Values were comparable to concurrent control values at the end of recovery.

TABLE 3.1.1.4
NOTEWORTHY FINDINGS FOR CLINICAL CHEMISTRY PARAMETERS

Parameter	Dose (mg/kg/day)											
	Control		48:3		120:7.5		240:15		240:0		0:15	
	M	F	M	F	M	F	M	F	M	F	M	F
Urea (mg/dL) d34	15.7	17.7	-	-	47.7	41.6	64.4	54.3	56.0	37.1	-	-
Urea (mg/dL) d90	15.5	18.5	-	-	32.8	31.2	47.7	31.8	43.4	23.9	-	-
Creatinine (mg/dL) d34	0.23	0.28	-	-	0.28	0.36	0.33	0.36	0.31	-	-	-
Creatinine (mg/dL) d90	0.26	--	-	-	-	-	0.34	-	0.34	-	-	-
Potassium (mEq/L) d34	5.2	4.4	-	-	5.8	5.1	6.1	5.5	5.8	5.2	-	-
Potassium (mEq/L) d90	5.1	4.4	-	-	-	4.9	5.6	4.7	-	4.7	-	-
Phosphorus (mg/dL) d34	-	5.9	-	-	-	-	-	7.1	-	6.7	-	-
Phosphorus (mg/dL) d90	-	4.7	-	-	-	-	-	-	-	5.2		-

No entries (-) are made in low dose combination group because of no noteworthy findings.

Urinalysis: No significant changes.

Organ Weights: Statistically significant and nondose-dependent decreases in mean absolute and relative (to body and/or brain) heart and liver weights were noted for both sexes with all dose combinations and with valsartan alone relative to control. Decreased liver weights (both absolute and relative) were also noted with amlodipine alone. Lower heart and liver weights were still present in both sexes in the high dose combination and valsartan (liver weights for males) groups at the end of the recovery period. A dose-dependent increase in ovarian weights was noted with amlodipine alone or in combination with valsartan. The latter effect was also noted in recovery group animals receiving amlodipine only (Table 3.1.1.5).

TABLE 3.1.1.5
NOTEWORTHY FINDINGS FOR ABSOLUTE ORGAN WEIGHTS

Parameter	Dose (mg/kg/day)									
	48:3		120:7.5		240:15		240:0		0:15	
	M	F	M	F	M	F	M	F	M	F
Heart, 13 wk	-17.4	-13.4	-16.9	-13.0	-20.9	-13.4	-24.9	-15.4	5.7	3.5
Recovery	-	-	-	-	-11.9	-6.4	-12.0	-9.2	-6.9	-1.8
Liver	-4.6	-12.1	-10.0	-5.2	-13.3	-3.4	-17.8	-7.8	-4.2	-4.0
Recovery	-	-	-	-	-6.1	-5.2	-7.3	4.4	-6.7	-9.5
Ovary		3.3		6.0		36.6		-10.4		12.9
Recovery		-		-		-3.2		-3.0		15.0

Number indicates mean percent difference from control.

Gross Pathology: Multiple dark foci in the stomach were noted in one high dose combination animal (#4011) sacrificed on day 38. These foci reflected marked severity of erosion and ulceration in the glandular stomach.

Histopathology: Main histopathological findings considered directly related to treatment were noted in the kidneys, stomach and bone marrow (the latter two findings in valsartan and valsartan/amlodipine combination groups). In kidneys, non dose-dependent increased incidence and severity (relative to control) of focal tubular basophilia/hyalinization, tubular dilatation, tubular casts (male only) and interstitial lymphocytic inflammation were noted in all dose groups. Males were distinctly more affected than females. Furthermore, arteriolar medial hypertrophy (characterized by medial thickening of small to medium-sized arterioles particularly those in close proximity to glomeruli) were present in both sexes given valsartan:amlodipine combinations of 120:7.5 or more mg/kg/day or valsartan alone at 240 mg/kg/day. These renal changes were also present in the recovery group. The sponsor attributes them to the expected blood pressure lowering effects of valsartan. Gastric irritation (inflammation, erosion/ulcer) of the glandular stomach was noted in both sexes treated with valsartan alone or in combination with amlodipine at all doses (except for inflammation in females at 48:3 mg/kg/day) and was not observed in the high dose combination recovery group. Stomach lesions were attributed to the valsartan component. Males were more affected than females. Additional histopathological findings included reduced bone marrow erythropoiesis (not noted in recovery group animals) in both sexes in the high dose combination and valsartan alone groups and hypertrophy of the zona glomerulosa (effect also noted in recovery group males) in the amlodipine only group. These effects, according to the sponsor, are pharmacological side effects of valsartan and amlodipine, respectively.

TABLE 3.1.1.6
SELECTED TREATMENT-RELATED MICROSCOPIC FINDINGS FOR BOTH SCHEDULED AND UNSCHEDULED SACRIFICES

Parameter	Dose (mg/kg/day)											
	Control		48:3		120:7.5		240:15		240:0		0:15	
	M	F	M	F	M	F	M	F	M	F	M	F
No of animals	10	10	10	10	10	10	11	11	10	10	10	10
(Postdose Recovery evaluated)	(6)	(6)					(5)	(5)	(6)	(6)	(6)	(6)
Adrenal	0	0	0	0	0	0	0	0	0	0	9	6
Hypertrophy, Z. Glom											(3)	
Bone marrow	0	0	0	0	0	0	6	3	2	7	0	0
↓ Erythropoiesis												
Kidney	1	0	2	0	10	3	9	2	9	0	3	0
Hyalinization, tubular							(3)	(2)	(4)			
Basophilia, tubular	6	2	5	2	10	9	10	11	10	9	6	2
	(4)	(3)					(5)	(5)	(6)	(5)	(4)	(2)
Inflammation lymphocytic	5	3	7	3	10	8	10	8	9	6	1	3
	(3)	(0)					(5)	(4)	(6)	(1)	(1)	(3)
Hypertrophy arteriole	0	0	0	0	10	8	9	9	6	6	0	0
							(3)	(4)	(2)	(3)		
Tubular casts	2	3	0	1	2	0	3	0	2	0	2	3
	(2)						(2)	(4)	(2)	(1)	(1)	(1)
Dilatation tubular	0	0	2	0	10	4	7	2	3	0	0	0
							(2)	(2)	(3)			
Stomach	0	0	0	0	3	1	3	4	2	0	0	0
Erosion glandular												
Inflammation, mixed	0	0	6	0	8	1	8	4	8	3	0	0
Ulcer glandular	0	0	0	0	0	0	1	1	0	0	0	0

Toxicokinetics: Based on dose normalized AUC values, exposures to the individual drug components increased with increase in dose but were not dose proportional. No tendency for accumulation was detected between day 1 and week 10 for either sex (Tables 3.1.1.7 and 3.1.1.8). Maximum plasma concentration was reached between 0.5 and 2 hr for valsartan and between 0.5 and 6 hr for amlodipine. Based on AUC or Cmax values, the exposure to amlodipine after dosing alone or in combination with valsartan was higher (1.6 to 3.4-fold) in week 10 than on day 1 for both sexes (Table 3.1.1.8). A similar phenomenon was not noted with the exposure to valsartan. Overall, amlodipine had no effect on valsartan exposure.

TABLE 3.1.1.7
13 WEEK TOXICITY STUDY IN RATS
MEAN TOXICOKINETIC PARAMETERS FOR VALSARTAN IN RAT PLASMA

Groups	Group 2		Group 3		Group 4		Group 5	
Treatment	Low combination		Mid combination		High combination		valsartan	
Sex	M	F	M	F	M	F	M	F
				Day 1/2				
t_{max} (h)	1	1	2	0.5	0.5	1	2	1
C_{max} (µg/mL)	11.7	12.1	19.1	15.6	27.3	26.5	23.6	29.9
C_{max}/Dose (µg/mL)/(mg/kg/day)	0.244	0.252	0.159	0.130	0.114	0.110	0.0983	0.125
AUC_{0-24h} (µg.h)/mL	62.9	58.5	398	71.0	226	134	179	164
AUC_{0-24h}/Dose ((µg.h)/mL)/(mg/kg/day)	1.31	1.22	3.31	0.592	0.942	0.558	0.745	0.684
				Week 10				
t_{max} (h)	1	0.5	1	1	0.5	1	2	0.5
C_{max} (µg/mL)	12.9	67.3	20.6	170	40.8	19.7	22.4	133
C_{max}/Dose (µg/mL)/(mg/kg/day)	0.269	1.40	0.172	1.42	0.170	0.0821	0.0933	0.554
AUC_{0-24h} (µg.h)/mL	39.5	88.1	143	265	193	152	286	191
AUC_{0-24h}/Dose ((µg.h)/mL)/(mg/kg/day)	0.822	1.84	1.19	2.21	0.804	0.633	1.19	0.794

TABLE 3.1.1.8
13 WEEK TOXICITY STUDY IN RATS
MEAN TOXICOKINETIC PARAMETERS FOR AMLODIPINE IN RAT PLASMA

Gender	Day	Treatment	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng*h/mL)	C_{max}/Dose (ng/mL/ mg/kg/day)	AUC_{0-24}/Dose (ng*h/mL/ mg/kg/day)
Male	Day 1	Low combination	48:3	31.2	6	421	10.40	140.29
		Mid combination	120:7.5	42.6	2	611	5.68	81.50
		High combination	240:15	117	6	2065	7.80	137.65
		High amlodipine	0:15	188	1	2884	12.53	192.27
	Week 10	Low combination	48:3	58.9	1	791	19.63	263.54
		Mid combination	120:7.5	137	6	2088	18.27	278.39
		High combination	240:15	392	6	5886	26.13	392.37
		High amlodipine	0:15	380	2	5908	25.33	393.83
Female	Day 1	Low combination	48:3	45.7	1	566	15.23	188.58
		Mid combination	120:7.5	105	6	1432	14.00	190.89
		High combination	240:15	127	6	1930	8.47	128.69
		High amlodipine	0:15	225	1	3186	15.00	212.38
	Week 10	Low combination	48:3	179	0.5	1170	59.67	390.09
		Mid combination	120:7.5	442	1	2350	58.93	313.36
		High combination	240:15	441	6	6445	29.40	429.66
		High amlodipine	0:15	580	1	5781	38.67	385.42

3.1.2. 13 Week Follow-Up Oral Gavage Study in Male S-D Rats

Key Study Findings: Gavage administration of valsartan and amlodipine at 48:3 mg/kg/day increased incidence and severity of inflammation and focal edema in the stomach. Mucosal erosion was noted in the glandular stomach of a rat in this dose group. The NOAEL for this study was 16:1 mg/kg/day.

Study No.: 0570345
Location of Report: EDR
Conducting Laboratory and Location: [redacted]

Dates of Study: Dosing was initiated on December 14, 2005 and terminal necropsies were begun on March 17, 2006
GLP Compliance: Yes
QA'd Report: yes (X) no ( )
Drug, Lot #: Amlodipine besylate, batch #TY003F03, [redacted] pure; Valsartan, batch #C0657, [redacted] pure
Formulation: The drugs were suspended in 0.5% (w/v) Klucel aqueous solution in weeks 1, 5 and 13.
Animals

Species/Strain: Rats, Sprague Dawley (Crl:CD(SD)IGSBR, from Charles River)
#/Sex/Group: 10 males/group.
Age: 8 weeks old at initiation of dosing
Weight: 256.2-331.2 gm, at initiation of dosing
Husbandry: Animals were housed in pairs, except during the urine collection period. Food and water were available *ad libitum* except for study defined fasting procedures and during the urine collection period.

Dosing

Doses: Valsartan and amlodipine were administered (at a ratio of 16:1) at doses of 0:0, 4:0.25, 16:1 or 48:3 mg/kg/day. Neither valsartan nor amlodipine were given alone to rats in this study. The doses were selected based on results of a 13 week study described in previous section where inflammation of the stomach was observed in males at 48:3 mg/kg/day (in females at 120:7.5 mg/kg/day). A NOAEL was not achieved in that study.
Route, Mode and Duration of Administration: Orally by gavage (5 ml/kg), once daily, for 13 weeks. Control animals received the vehicle.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for clinical signs and mortality.
Body Weight and Food Consumption: Recorded once before treatment and once weekly during the dosing period.
Ophthalmology: Conducted prior to initiation of dosing on all animals and in week 13 on all surviving control and high dose animals only.
Hematology and Clinical Biochemistry: Blood samples were collected from the sublingual vein of all surviving animals in weeks 5 and 13 for hematology (erythrocytes, hematocrit, hemoglobin, Wintrobe indices, red cell distribution width, reticulocytes, white blood cell count, white blood cell differential, platelets) and blood chemistry (ALT, AST, AP, total bilirubin, total protein, albumin, globulins, glucose, urea, creatinine,

creatine kinase, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, cholesterol, A/G ratio) examinations.
Urinalysis: Urine samples were collected from individual animals for up to 5 hr and analyzed for specific gravity, bilirubin, blood, glucose, ketones, protein, urobilinogen, pH). Food and water were removed during collection.
Pathology: Animals were fasted overnight prior to terminal necropsy and weighed before sacrifice. A complete necropsy was conducted on all animals with a recording of macroscopic observations for all tissues listed in Table 3.1.2.1. Organ weights were recorded only at scheduled sacrifice. However, the data is not reported. Immediately after weighing, samples from the glandular stomach were frozen for possible investigational gene expression analysis[1]. Microscopic examinations were performed on all tissues listed in Table 3.1.1.2 from all animals in all groups. However, the report includes data for only kidney and stomach.
Toxicokinetics: Blood samples were collected from the sublingual vein under light isoflurane anesthesia on day 1 and in week 11 at 0.5, 1, 2, 6 and 24 hr after dosing (2 rats/group/time point).

TABLE 3.1.2.1

Tissue list: for collection, weighing (W), processing (P) and/or genomics (G)

W	adrenal				pancreas
	aorta				parathyroid
	bone marrow (in bone)	W			pituitary
W	brain	W			prostate
	cecum				rectum
	colon				salivary gland
	duodenum				sciatic nerve
	epididymis				seminal vesicle
	esophagus				skeletal muscle
	eye				skin
	femur/tibia				spinal cord
	harderian gland	W			spleen
W	heart				sternum
	ileum		P	G	stomach
	jejunum	W			testis
W	kidney				thymus
	lacrimal gland	W			thyroid
W	liver				tongue
	lung				trachea
	lymph node - bronchial				urinary bladder
	lymph node - mandibular		P		macroscopic lesions
	lymph node - mesenteric				animal identification
	mammary gland area				
	nasal passage				

[1] Gene expression analyses were not considered a part of this study and thus were not included in this report.

Results

Analysis of Formulations: The formulation was stable for at least 12 days at 6°C and for at least 4 hr at room temperature. Mean concentrations of all samples analyzed were in the range of 91% to 112% of target concentrations.

Mortality: No mortality occurred in the study

Clinical Signs: No test substance-related clinical signs noted during the study.

Body Weights: No treatment-related effects were noted on body weight parameters at combination doses of 4:0.25 and 16:1 mg (valsartan:amlodipine)/kg/day. However, at the high combination dose (48:3 mg/kg/day), non-significant reductions from control mean body weight gain were noted on study day 8 (a 15% decrease) with reductions continuing throughout the study (8-12% on all other days).

Food Consumption: No treatment-related findings.

Ophthalmoscopy: No significant ocular changes

Hematology, Clinical Chemistry and Urinalysis: There were no changes relative to control group.

Gross Pathology: No treatment-related findings.

Histopathology: Test substance-related effects were confined to the stomach in the high dose combination group. An increased incidence and severity of inflammation and focal edema were noted in the stomachs of rats receiving 48:3 mg/kg/day. Mucosal erosion was noted in the glandular stomach of a rat (1/10) in this dose group (Table 3.2.2). The inflammation consisted of an admixture of neutrophils, lymphocytes and eosinophils.

TABLE 3.1.2.1
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS IN STOMACH

SEX :				
DOSE GROUP:	1	2	3	4
NO.ANIMALS:	10	10	10	10
STOMACH :	10	10	10	10
N.A.D. :	-	1	1	-
- Inflammation,Mixed :	8	9	7	10
Grade 1:	2	3	4	1
Grade 2:	5	4	2	6
Grade 3:	1	2	1	3
- Dilat.,Mucosal Gland:	6	7	6	7
Grade 1:	6	6	6	7
Grade 2:	-	1	-	-
- Vacuolation,Cytopl. :	-	-	-	2
Grade 1:	-	-	-	2
- Necrosis,Single Cell:	1	-	-	1
Grade 2:	1	-	-	1
- Erosion,Gl.Stomach :	-	-	-	1
Grade 1:	-	-	-	1
- Edema,Gl.Stomach :	-	-	-	3
Grade 1:	-	-	-	1
Grade 2:	-	-	-	2
- Edema,Non-Gl.Stomach:	2	1	1	-
Grade 1:	2	1	1	-
- Hyperplasia,Mucosal :	-	-	-	1
Grade 1:	-	-	-	1

Groups 1, 2, 3 and 4 are, respectively, Control, 4:0.25, 16:1 and 48:3 (valsartan: amlodipine) mg/kg/day.

Toxicokinetics: Based on the mean dose-normalized AUC values, exposures to valsartan and amlodipine increased with increase in dose but were not dose proportional. The plasma exposure (AUC) to amlodipine was higher during week 11 than in week 1 for all treated dose groups. In contrast, it was lower for valsartan (Table 3.1.2.2). Maximum plasma concentration (Cmax) for valsartan was reached between 0.5 and 1 hr, while Cmax for amlodipine was reached between 2 and 6 hr. Overall, amlodipine had no effect on valsartan exposure.

TABLE 3.1.2.2
TOXICOKINETICS OF VALSARTAN AND AMLODIPINE AFTER 13 WEEKS ORAL DOSING IN MALE RATS

Valsartan							
		Group 2		Group 3		Group 4	
Dose Valsartan /amlodipine		4:0.25 mg/kg/day		16:1 mg/kg/day		48:3 mg/kg/day	
Parameter	Units	Day 1/2	Day 72/73	Day 1/2	Day 72/73	Day 1/2	Day 72/73
t_{max}	h	1	0.5	0.5	0.5	1	1
C_{max}	ng/mL	1130	1280	5320	3780	10700	13500
C_{max}/Dose	(ng/mL)/(mg/kg/day)	283	320	333	236	223	281
AUC_{0-24h}	ng*h/mL	5050	4130	21600	16600	63100	53300
AUC_{0-24h} /Dose	(ng*h/mL)/(mg/kg/day)	1260	1030	1350	1040	1310	1110
Amlodipine							
		Group 2		Group 3		Group 4	
Dose Valsartan /amlodipine		4:0.25 mg/kg/day		16:1 mg/kg/day		48:3 mg/kg/day	
Parameter	Units	Day 1/2	Day 72/73	Day 1/2	Day 72/73	Day 1/2	Day 72/73
t_{max}	h	6	6	6	2	2	2
C_{max}	ng/mL	1.28	2.04	6.49	15.4	15.5	48.3
C_{max}/Dose	(ng/mL)/(mg/kg/day)	5.12	8.16	6.49	15.4	5.17	16.1
AUC_{0-24h}	ng*h/mL	17.2	28.1	91.1	180	209	518
AUC_{0-24h} /Dose	(ng*h/mL)/(mg/kg/day)	68.8	112	91.1	180	69.7	173

3.1.3 13 Week Oral Gavage Study in Marmosets With a 4 Week Recovery

Key Study Findings: Oral gavage administration of valsartan and amlodipine besylate produced severe clinical signs (160:10→80:5 mg/kg/day), reduced food consumption and body weight loss (≥40:2.5 mg/kg/day) and resulted in moribundity due to erosive/ulcerative inflammation of the cecum and colon (160:10 mg/kg/day). Test substance-related microscopic pathology was identified in kidney, atria and large intestine (160:10→80:5 mg/kg/day). Inflammation of the large intestine and atria was noted in recovery animals. A NOAEL was not determined in this study.

Study No.: 0570032
Location of Report: EDR
Conducting Laboratory and Location: [redacted]

Dates of Study: The animals were dosed on March 9, 2005 and necropsied between June 9 and July 12, 2005
GLP Compliance: Yes
QA'd Report: yes (X) no ( )
Drug, Lot #: Amlodipine besylate, batch #TY003F03, [redacted] valsartan, lot #1030765000 and C0657, [redacted]
Formulation: The drugs were suspended in 0.5% (w/v) Klucel aqueous solution prepared for dosing in weeks 1, 3, 5 and 13.
Animals

Species/Strain: Marmoset (*Callithrix jacchus*)
#/Sex/Group: 3/sex/group. An additional 2 animals each were included for the control, high dose combination, valsartan and amlodipine alone groups to serve as recovery animals for a 4 week recovery period. See Table 3.1.3.1 for details.
Age: 1.5 to 6 years old at start of study
Weight: males: 230.0 to 433.8 gm, females: 299.9 to 492.9 gm at start of dosing
Husbandry: Animals were housed individually in primate cages. Food and water, *ad libitum*, were given throughout the study period.

Dosing

Doses: Valsartan and amlodipine were administered (at a ratio of 16:1) at doses of 0:0, 40:2.5, 80:5 or 160:10 mg/kg/day. Two additional groups of marmosets received either valsartan or amlodipine at 160 or 10 mg/kg/day, respectively (Table 3.1.3.1). Following nine doses at 160:10 mg/kg/day, dosing was suspended from study days 10 to 14 (washout period) due to severe clinical signs and was resumed on study day 15 with the high dose combination dose reduced to 80:5 mg/kg/day. A similar change was made to the dosage regimen for the valsartan and amlodipine alone groups on study day 15 (after 5 days washout) when the high dose was decreased to 80 and 5 mg/kg/day, respectively (see footnote to the Table 3.1.3.1). The doses were selected on the basis of a 2 week oral study in marmosets in which gavage administration of valsartan and amlodipine besylate at doses of 480 mg valsartan and 30 mg amlodipine/kg/day resulted in the sacrifice of a moribund female on day 15 due to severe clinical signs, excessive body weight loss and pathological changes suggestive of renal failure. Animals receiving 240:15 or more mg/kg/day showed right atrial hemorrhage, edema, inflammation and exacerbation in the

severity of renal disease. Hypertrophy of the adrenal cortex was noted at all combination doses (80:5 or more mg/kg/day) and in animals treated with valsartan or amlodipine besylate alone.

Route, Mode and Duration of Administration: Orally by gavage (5 ml/kg), once daily, for 13 weeks. Recovery animals were also treated for 13 weeks but were not killed until 4 weeks later.

TABLE 3.1.3.1
STUDY DESIGN

		Animal Numbers		Dose	Concentration
				Valsartan: amlodipine base (salt)	Valsartan: amlodipine salt
Group	Number/sex	males	females	(mg/kg/day)	(mg/mL)
1	3	1001-1003	1501-1503	0	0
	+2 recovery	1004, 1005	1504, 1505		
Control					
2	3	2001-3	2501-3	40:2.5 (3.5)	8:0.7
Low Combination					
3	3	3001-3003	3501-3503	80:5 (6.9)	16:1.4
Mid Combination					
4*	3	4001-4003	4501-4503	160:10 (13.9) → 80:5 (6.9)	32:2.8 → 16:1.4
High Combination	+2 recovery	4004-4005	4504-4505		
5*	3	5001-5003	5501-5503	160 → 80	32 → 16
High Valsartan	+2 recovery	5004-5005	5504-5505		
6*	3	6001-6003	6501-6503	10 (13.9) → 5 (6.9)	2.8 → .1.4
High Amlodipine	+2 recovery	6004-6005	6504-6505		

Salt/base ratio for amlodipine besylate is 1.387.

*Dosing was suspended in group 4, 5 and 6 animals on days 10-14 and resumed on day 15 at doses equivalent to the mid dose combination animals.

Observations and Measurements

Clinical Signs: All animals were observed at least twice a day for mortality, moribundity and clinical signs.

Body Weights: Recorded for all animals before treatment on the first day of dosing and then twice weekly during weeks 1-5, and weekly thereafter until necropsy or until the end of recovery period for those animals in the recovery phase.

Food Consumption: Estimated daily from the beginning of the pretest period to the end of the recovery period.
Ophthalmoscopy: Conducted on all animals prior to the initiation of dosing and in week 12. Not performed during recovery period.
ECG: Recorded from all study animals pre-dose and from all surviving animals in study weeks 4, 5, 8 and 12 approximately 1.5 to 2.5 hr post dose. Also recorded in surviving recovery animals in week 4.
Hematology and Clinical Chemistry: Blood samples were collected from all (non-fasted) study animals pretest and during treatment weeks 5, 6 and 13, and during recovery week 4 for hematology (erythrocytes, hematocrit, hemoglobin, prothrombin time, activated partial thromboplastin time, Wintrobe indices, red cell distribution width, reticulocytes, white blood cell count, white blood cell differential, platelets) and blood chemistry (ALT, AST, AP, total bilirubin, total protein, albumin, globulins, glucose, urea, creatinine, creatine kinase, sodium, potassium, chloride, calcium, inorganic phosphorus, magnesium, triglycerides, cholesterol, A/G ratio) examinations..
Urinalysis: Urine samples were collected from individual animals pretest and during treatment weeks 5, 6 and 13 and during recovery week 4 for up to 3 hr after dosing. The following parameters were assessed: specific gravity, bilirubin, blood, glucose, ketones, protein, urobilinogen, pH.
Pathology: Animals were fasted overnight (18 hr) prior to terminal necropsy and weighed before sacrifice. A complete necropsy was conducted on all animals with a recording of macroscopic observations for all tissues listed in Table 3.1.3.2. At schedule sacrifices, 1 ml blood was collected for genomics from all surviving animals. Organ weights were recorded only at scheduled sacrifices. Immediately after weighing, samples from the liver, kidney, spleen, heart (apex), thymus, skeletal muscle, ileum, jejunum and aorta (thoracic and aortic arch regions) were frozen for possible investigational gene expression analysis[2]. Microscopic examinations were performed on all tissues listed in Table 3.1.3.2 from all animals. All gross lesions and target organs (kidneys, adrenal, heart, liver, cecum, colon and rectum) were processed for microscopic evaluation from all animals in recovery groups.
Toxicokinetics: Blood samples were collected from all non-recovery study animals at 1, 3, 8 and 24 hr after dosing during study weeks 1 (days 1, 2) and 10.

[2] Gene expression analyses were not considered part of this study and thus were not included in this report.

TABLE 3.1.3.2

Tissue list for collection, weighing (W) and/or processing (P)

W	P	adrenal		P	pancreas
	P	aorta		P	parathyroid
	P	bone marrow (in bone)	W	P	pituitary
W	P	brain	W	P	prostate
	P	cecum		P	rectum
	P	cervix		P	salivary gland
	P	colon		P	sciatic nerve
	P	duodenum		P	seminal vesicle
	P	epididymis		P	skeletal muscle (left leg)
	P	esophagus		P	skin
	P	eye		P	spinal cord
	P	femur (distal w/joint)	W	P	spleen
	P	gall bladder		P	sternum
W	P	heart		P	stomach
	P	ileum	W	P	testis
	P	jejunum		P	thymus
W	P	kidney	W	P	thyroid
	P	lacrimal gland		P	tongue
W	P	liver		P	trachea
	P	lung		P	urinary bladder
	P	lymph node – bronchial	W	P	uterus
	P	lymph node – mandibular		P	vagina
	P	lymph node – mesenteric		P	macroscopic lesions
	P	mammary gland			animal identification
W	P	ovary		P	bone marrow smear
	P	common carotid artery (left)			

Results

Analysis of Formulations: The formulation was stable for at least 12 days at 6°C and for at least 4 hr at room temperature. Mean concentrations of all samples analyzed were in the range of 91% to 114% of target concentrations.

Mortality: Three animals were sacrificed moribund or died between study days 8 and 35. A female (#4504) receiving 160:10 mg (valsartan:amlodipine)/kg/day was sacrificed in moribund condition on day 8. Test substance-related clinical signs were noted (following 3 doses) which included reduced food consumption on days 4-7, severe fecal changes (diarrhea, mucoid bloody feces) on days 5-8, thin appearance and 19% body weight loss on day 8, cold to touch and decreased locomotor activity on the day of sacrifice. The cause of moribundity was erosive large intestinal enteritis, which was considered test substance-related. A second female (#5502) receiving valsartan alone (160 mg/kg/day) was moribund and died just prior to necropsy on day 8. This animal exhibited test substance-related clinical signs of reduced food consumption (on days 5-8), fecal changes

(diarrhea with apparent blood and mucus on days 3-8), severe body weight loss on day 8 and was cold to touch. The cause of death was ulcerative large intestinal enteritis plus systemic amyloidosis. A male (#4002) receiving 160:10 → 80:5 mg/kg/day was sacrificed moribund on day 35. Clinical signs included reduced food consumption, body weight loss, decreased locomotor activity and pale gingiva. The cause of moribundity was multicentric malignant lymphoma and was not considered test substance-related.
Clinical Signs: Test article-related clinical signs (frequency and/or severity of soft feces and diarrhea, with blood in a few animals, emesis with feed, thin appearance) were confined to the high dose groups (160:10 → 80:5 mg/kg/day, 160 →80 mg valsartan/kg/day and 10 → 5 mg amlodipine/kg/day). During the recovery phase, the majority of clinical signs abated with the exception of thin appearance in one or two animals. No clinical signs were noted in other dose groups.
Body Weights: Treatment-related body weight loss relative to control or to baseline was noted for both sexes at all doses and the effect was more severe at the high combination dose 160:10 → 80:5 mg/kg/day and with valsartan and amlodipine alone. Individual low-dose combination animals exhibited weight losses (6- 9% for males, 11% for females) for the duration of the study, while all mid-dose combination animals exhibited weight losses (up to 16% for males and 9% for females) as early as day 10 of the study. At the high dose combination of 160:10 → 80:5 mg/kg/day, severe and rapid body weight losses (5-15%) occurred in females following the first 9 days of treatment. Following the dose reduction on day 15, the results were more variable, with some of the surviving animals gaining weight and others demonstrating continued weight loss. The valsartan and amlodipine alone groups also displayed test substance-related body weight losses with the amlodipine-treated males clearly exhibiting the most severe losses. During the recovery phase, the animals demonstrated weekly gains or improvement in body weight.
Food Consumption: Decreases in food consumption (up to 25% relative to control) were noted in individual animals at doses of 80:5 or more mg/kg/day and at 10 → 5 mg amlodipine/kg/day.
Ophthalmoscopy: There were no treatment-related ocular changes across groups.
ECG: No significant changes.
Urinalysis: There were no significant changes.
Hematology: A slight decrease in erythroid parameters was noted in individual animals of both sexes receiving 160 →80 mg valsartan/kg/day, with or without amlodipine at 10 → 5 mg/kg/day. Partial to full recovery was noted during the recovery period.
Clinical Chemistry: Mild to moderate elevation in BUN was noted in individual animals of both sexes receiving 160 →80 mg valsartan/kg/day, with or without amlodipine at 10 → 5 mg/kg/day. Reversibility could not be evaluated.
Organ Weights: Test substance-related decreased liver weights relative to control (33% absolute and 35% relative to body weight) was noted for males dosed with 10 →5 mg amlodipine/kg/day. A similar decrease was noted in recovery animals.
Gross Pathology: Macroscopic findings confined to a high dose combination (160:10 → 80:5 mg/kg/day) female (#4502) displaying enlarged, mottled kidneys.
Histopathology: Test substance-relating findings were noted in the kidney, heart, adrenal glands and large intestine (Table 3.1.3.3). A multifaceted nephropathy characterized by variable amounts of lymphocytic interstitial inflammation, fibrosis, tubular casts, dilatation and basophilia was noted in both control and treated animals. However, a slight

exacerbation in the severity of this renal disease was noted in two females, one receiving a high dose combination and another receiving valsartan alone. In addition, medial hypertrophy of renal cortical vessels were noted in these two animals. Though these were considered valsartan–related effects, spontaneous nephropathy is commonly seen in laboratory marmosets. Minimal to slight lymphocytic atrial myocardial inflammation was noted in individual animals at all dose levels, including one recovery male receiving amlodipine alone. This finding was attributed to the amlodipine component since a similar observation was made in an earlier study with amlodipine alone (NDA 19,787 review). Moderate multifocal vacuolation of the adrenal cortex (zona fasiculata) was seen in all males including control with a slight increase in severity relative to control in drug treated groups (Table 3.1.3.3). The sponsor, however, attributes the changes noted in the adrenal cortex to a spontaneous, non-specific, adaptive response. Moderate to marked erosive or ulcerative inflammation was noted in the large intestine (cecum and/or colon) in all groups including control females. Drug-treated groups exhibited increased incidence and severity (dose-dependent) relative to control. The intestinal lesions were considered to be amlodipine-related. One of these animals (#4504) was sacrificed moribund after one week of treatment, and its moribundity was considered to be treatment-related. Ulcerative inflammation of the large intestine was also noted in recovery animals (Table 3.1.3.3).

TABLE 3.1.3.3
SELECTED TREATMENT-RELATED MICROSCOPIC FINDINGS FOR BOTH SCHEDULED AND UNSCHEDULED SACRIFICES

Parameter	Dose (mg/kg/day)											
	Control		Valsartan: Amlodipine						160 → 80 Valsartan		10 → 5 Amlodipine	
			40:2.5		80:5		160:10→80:5					
	M	F	M	F	M	F	M	F	M	F	M	F
No of animals examined (Recovery evaluated)	3 (2)	3 (2)	3	3	3	3	3 (2)	4 (1)	3 (2)	3 (2)	3 (2)	3 (2)
Vacuolation adrenal cortex												
Z. fasiculata, grade 1			1		2		1				1	
Grade 2	2	-	2	-	-	-	1	-		-	-	-
Grade 3			-		1		-		3		2	
Kidney, nephropathy (exacerbation)	-	-	-	-	-	-	-	1^{+++}	-	1^{++}	-	-
Atrial inflammation (lymphocytic)	-	-	-	1^{+}	1^{+}	-	2^{++}	-	-	1^{+}	2^{+} (1^{++})	1^{+}
Cecum					1^{++}		1^{+++}					
Inflammation	-	2^{++} 1^{+++}	1++	1^{+++}	1^{+++} +	-	1^{++++} (2^{++})	1^{+++}	-	1^{+++} (1^{+++})	1^{++++} (2^{+++})	-
Erosion	-	-	-	-	1^{+++}	-	-	1^{++}	-	-	- (1^{++})	-
Ulcer	-	-	-	-	-	-	-	-	-	-	1^{+++} (1^{++})	1^{+++}
Colon							1^{+++}			1^{+++}	1^{++}	
Inflammation	-	1^{+++}	1^{+}	1^{+++}	2^{++}	-	2^{++++} (2^{++})	2^{++}	-	(1^{+++})	(2^{++})	-
Ulcer	-	-	-	-	-	-	1^{++++}	-	-	1^{+++}	-	-

- indicates no noteworthy findings.
+ grade 1 or minimal, ++ slight, +++ moderate, ++++ marked.

Toxicokinetics: Exposures to the individual drug components increased with increase in dose but were not dose proportional. There were no gender differences in exposure for either compound. Exposure to valsartan and amlodipine was the same whether the compounds were administered together or not, suggesting no effect of one on the absorption and disposition of the other. There was no accumulation seen with repeated dosing (Tables 3.1.3.4 and 3.1.3.5). The Cmax data showed considerable variability. Maximum plasma concentration for valsartan was reached between 1 and 3 hr, while that for amlodipine was reached between 3 and 8 hr.

TABLE 3.1.3.4
13 WEEK TOXICITY STUDY IN MARMOSETS
MEAN TOXICOKINETIC PARAMETERS FOR VALSARTAN IN RAT PLASMA

Dose*	Study Day	Gender	$AUC_{(0\text{-}24h)}$ (ng*h/mL)	C_{max} (ng/mL)	t_{max}(h)
40:2.5	1-2	Male	17700	2020	1
		Female	11800	2130	1
	69-70	Male	44400	8570	3
		Female	11000	2560	1
80:5	1-2	Male	55600	3880	8
		Female	19200	3640	1
	69-70	Male	31800	8280	1
		Female	26200	4800	1
160:10	1-2	Male	93900	16300	3
		Female	83000	4120	3
80:5**	69-70	Male	27600	5390	1
		Female	32100	3490	3
160:0	1-2	Male	90100	8130	1
		Female	55200	6960	1
80:0**	69-70	Male	37600	7840	1
		Female	50600	9700	3

*Dose expressed as valsartan:amlodipine (mg/kg/day)

**Dose lowered on day 15

TABLE 3.1.3.5
13 WEEK TOXICITY STUDY IN MARMOSETS
MEAN TOXICOKINETIC PARAMETERS FOR AMLODIPINE IN MARMOSET PLASMA

Dose*	Study Day	Gender	$AUC_{(0\text{-}24h)}$ (ng*h/mL)	C_{max} (ng/mL)	t_{max}(h)
40:2.5	1-2	Male	300.25	24.1	8
		Female	35.35	10.1	3
	69-70	Male	270.60	19.5	8
		Female	82.60	23.6	8
80:5	1-2	Male	157.27	42.6	8
		Female	540.05	42.5	8
	69-70	Male	60.90	17.4	3
		Female	346.15	31.1	3
160:10	1-2	Male	1427.90	123	8
		Female	1264.52	70.5	8
80:5**	69-70	Male	399.35	33.2	8
		Female	408.05	19.4	8
0:10	1-2	Male	2812.35	322	3
		Female	1633.10	109	8
0:5**	69-70	Male	1199.70	143	3
		Female	1237.30	84.8	3

*Dose expressed as valsartan:amlodipine (mg/kg/day)

**Dose lowered on day 15

3.2. Reproductive Toxicology

3.2.1. An Oral Embryo-fetal Development Study in S-D Rats

Key Study Findings: Gavage administration of valsartan and amlodipine besylate did not result in any evidence of teratogenic potential. However, evidence of maternal toxicity (significant decreases in body weight gain and food consumption relative to control) was noted at 160:10 or more mg/kg/day and at 20 mg amlodipine/kg/day. At 320:20 mg (valsartan:amlodipine)/kg/day, the highest combination dose administered, developing fetuses displayed decreased body weight. The NOAELs for maternal and fetal effects were, respectively, 80:5 and 160:10 mg (valsartan:amlodipine)/kg/day.

Study No.: 0470007
Location of Report: EDR
Conducting Laboratory and Location: [redacted]

Dates of Study: The animals were dosed between February 8 and 17, 2004 and c-sectioned between February 23 and March 3, 2004.
GLP Compliance: Yes
QA'd Report: yes (X) no ( )
Drug, Lot #: Amlodipine besylate, batch #TY003F03, [redacted] pure; Valsartan, lot #1010184000 and 1030765000, [redacted] pure
Formulation: The drugs were suspended in 0.5% (w/v) Klucel aqueous solution. The frequency of preparation is not given.
Animals

Species/Strain: Rats, Sprague Dawley (Crl:COBS CD[SD]BR), from Charles River
#/Group: 25 presumed pregnant females/group. An additional 6 animals were included with each group (4 for the control), to serve as satellites for toxicokinetic study.
Age: 11 to 12 weeks old at start of study
Weight: 225 to 250 gm on gestation day 0
Husbandry: Housed individually in cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Suspensions of valsartan and amlodipine besylate were administered (at a valsartan:amlodipine ratio of 16:1) orally by gavage (10 ml/kg), once daily, to groups of presumed pregnant females at doses of 0:0, 80:5, 160:10, or 320:20 mg/kg/day. Two additional groups of rats received either valsartan or amlodipine at 320 or 20 mg/kg/day, respectively (Table 3.2.1.1). The control animals received the vehicle (10 ml/kg body weight). All animals were treated from gestation day 6 to gestation day 17. The doses were selected on the basis of a 2 week dose range-finding study in male and non-pregnant female IGS Wistar Hannover rats (a strain different from that used for the current study) in which gavage administration of 320:40 mg (valsartan:amlodipine)/kg/day (8:1 ratio) resulted in severe clinical signs and deaths of 2 males and 3 females between days 4 and 8. The dose was reduced on treatment day 6 to 240:30 mg/kg/day. Dosing was suspended for these animals on day 7 and all animals in this group (5/sex) were necropsied on day 8. Three females receiving 40→30 mg amlodipine/kg/day also died during the same

interval. The cause of death in all cases was the result of esophageal or nonglandular stomach ulcers and was attributed to the amlodipine component of the formulation. Decreased body weight gain was noted for animals receiving 80:10 or more mg/kg/day.

TABLE 3.2.1.1
STUDY DESIGN, ANIMAL ALLOCATION AND TEST ARTICLE DOSES

Group	Number/group main study	Satellites	Animal numbers[a,b] main study	satellites	Dose (mg/kg/day)	Concentration (mg/mL)*
1 Control	25	4	1-49	701-707	0	0
2 Low	25	6	55-103	713-723	80:5[c]	8:0.5[c]
3 Mid	25	6	109-157	729-739	160:10[c]	16:1[c]
4 High	25	6	163-211	745-755	320:20[c]	32:2[c]
5-High Valsartan	25	6	217-265	761-771	320	32
6-High Amlodipine	25	6	271-319	777-787	20	2

[a]PSA computer assigns odd numbers to females.

[b]To facilitate computer processing, a separate study was set up on the computer (study no. 0470007T) for processing the satellite females utilized for toxicokinetic measurements.

[c]Indicates Valsartan : amlodipine dose or concentration.

*The concentrations of amlodipine besylate were not corrected for salt/base ratio

Observations and Measurements

Clinical Signs: All main study animals were observed twice daily for general condition, mortality and moribundity. Clinical signs were not recorded for satellite animals.

Body Weights: Individual body weights were recorded on gestation days 0, 3, 6, 9, 12, 15,18 and 21 for main study animals and on gestation days 0, 3, 6, 9, 12, 15 and 18 for satellite animals.

Food Consumption: Recorded for main study animals on gestation days 3, 6, 9, 12, 15, 18 and 21.

Laparotomy: All surviving maternal main study and toxicokinetic satellite animals were euthanized on gestation day 21 and gestation day 18, respectively, and the major viscera (including placenta) were macroscopically observed. No tissues were saved for histopathologic examination. The gravid uterus was excised and weighed. The numbers of corpora lutea, resorptions, live and dead fetuses were recorded for main study animals. Each viable fetus obtained on gestation day 21 was sexed, weighed, examined externally for gross abnormalities and then sacrificed. Visceral examination was performed on approximately on-half of the fetuses from each litter. The remaining fetuses were placed in 70% ethanol for skeletal examinations. Fetal findings were classified as variations or malformations.

Toxicokinetics: Maternal blood samples were collected from the retro-orbital venous plexus of satellite animals following dosing on gestation day 17 at 1, 2, 8 and 24 hr (each animal was bled twice).

Results

Analysis of Formulations: The formulation was stable for at least 12 days at 6°C and for at least 4 hr at room temperature. Mean concentrations of all samples analyzed were within 93% to 114% of target concentrations.

Mortality: There were no deaths in this study.

Clinical Signs: No clinical signs were seen except for 3 females in the high dose combination group on gestation day 21. The signs included rough coat appearance, decreased stool, red stains in the pan and cool to touch. Since these signs were seen 4 days after the dosing period ended, the sponsor suggests that they were indicative of the start of labor/delivery and not a direct effect of the test substance. One female receiving valsartan alone (320 mg/kg/day) delivered just prior to scheduled c-section on the morning of gestation day 21.

Body Weights: Treatment-related reductions in body weight gain relative to control were noted at doses of 160:10 or more mg/kg/day and with valsartan or amlodipine alone at 320 or 20 mg/kg/day, respectively (Table 3.2.1.2). Statistically significant reductions in body weight gain relative to control were noted for gestation days 6 to 12 at 160:10 mg/kg/day and from gestation days 6 to 21 at 320:20 mg/kg/day. Statistically significant reductions were also noted at 20 mg amlodipine/kg/day for gestation days 6 to 9 and 15 thorough 18 (Table 3.2.1.2).

TABLE 3.2.1.2
MEAN GESTATION BODY WEIGHT CHANGE (GRAM)

	DOSAGE	0 MG/KG	80:5 MG/KG	160:10 MG/KG	320:20 MG/KG	320MG/KG VALSAR	20 MG/KG AMLODIP
DAYS 0 TO 3	MEAN	12	15	15	12	15	13
	S.D.	8	7	8	5	9	6
	N	25	24	24	25	24	25
DAYS 3 TO 6	MEAN	20	20	20	19	22	19
	S.D.	6	6	6	5	5	5
	N	25	24	24	25	24	25
DAYS 6 TO 9	MEAN	14	10	7**	8**	14	4**
	S.D.	5	5	6	6	5	9
	N	25	24	24	25	24	25
DAYS 9 TO 12	MEAN	21	18	15*	15*	18	20
	S.D.	5	9	8	5	8	12
	N	25	24	24	25	24	25
DAYS 12 TO 15	MEAN	23	22	18	14**	18	21
	S.D.	5	4	9	7	6	12
	N	25	24	24	25	24	25
DAYS 15 TO 18	MEAN	38	36	33	26**	32	23**
	S.D.	7	7	8	13	5	13
	N	25	24	24	25	24	25
DAYS 18 TO 21	MEAN	61	62	69	49**	62	71*
	S.D.	8	10	8	24	9	10
	N	25	24	24	25	24	25

Statistical key: * = p<0.05 ** = p<0.01

Food Consumption: A reduction in food consumption relative to control was noted at doses of 160:10 or more mg/kg/day and with valsartan or amlodipine alone. However, differences from control were significant only for the high combination dose group for gestation days 6 through 18 (decreases ranged from 14 to 22%).

Laparotomy: There were no test substance-related effects on reproductive parameters, maternal necropsy findings, fetal sex ratios or fetal external appearance at any dose level. Fetal weights were slightly but significantly decreased (5% relative to control weights) at

the high combination dose. An increased incidence of dilated ureters ($p < 0.05$) was noted at the high combination dose (statistically significant on fetal basis but not on litter basis) and at 320 mg valsartan/kg/day (statistically significant on both fetal and litter basis). Misshapen sternebrae and unossified forepaw phalanges, noted at increased incidence in the high dose combination group fetuses (neither finding significant on a litter basis), were considered indicative of developmental delays, the slight decrease in fetal weight and significant maternal toxicity. The dilated ureters, unossified forepaw phalanges and misshapen sternebrae are considered to be variations, common findings in this species and strain. The incidence of these variations was increased by valsartan. Similar findings were noted in an earlier study with valsartan alone (NDA 20,665 review).

TABLE 3.2.1.3
EMBRYO-FETAL TOXICITY STUDY IN RATS
SUMMARY OF CESAREAN DATA (GESTATION DAY 21) AND FETAL EXAMINATION DATA

Daily dose (mg/kg)	0 (Control)	80:5 (Low)	160:10 (Mid)	320:20 (High)	320 Valsartan	20 Amlodipine
Females, pregnant	25	24	24	25	24	25
Premature delivery	0	0	0	0	1	0
With viable fetuses	25	24	24	25	23	25
With all resorptions	0	0	0	0	0	0
Mean no. corpora lutea	16.5	16.6	16.5	15.0	16.4	16.3
Mean no. implantations	14.8	14.8	14.7	13.7	14.3	14.7
Mean % preimplantation loss	10.2	11.1	11.1	9.0	13.2	9.8
Litters						
No. litters evaluated	25	24	24	25	23	25
No. live fetuses	14.1	13.8	14.0	13.2	13.6	13.8
Mean no. resorptions	0.7	0.9	0.7	0.5	0.7	0.8
Litters with dead fetuses	0	0	0	0	0	0
% Postimplantation loss	4.9	6.2	4.5	3.5	4.6	5.7
Mean fetal body weight (g)	5.7	5.8	5.8	5.4*	5.9	5.7
Fetal sex ratios (% males)	53.5	51.5	52.5	47.0	48.6	51.7
Fetal malformations (litters)						
Gross external malformations	-	-	-	-	-	-
Visceral malformations	-	-	-	-	-	-
Skeletal malformations	-	-	-	-	-	-
Fetal variations (litters)	-	-	-	-	-	-
Gross external variations						
Visceral variations,	0 (0)	2 (2)	1 (1)	5* (3)	6** (4*)	3 (2)
total variations	0 (0)	2 (2)	1 (1)	5* (3)	6** (4*)	3 (2)
dilated ureter						
Skeletal variations (litters)	152 (25)	106**(21)	120**(23)	138 (23)	118*(23)	122**(23)
total variations	14 (7)	7 (5)	13 (9)	34** (11)	12 (6)	12 (6)
forepaw phalanx unossified	4 (3)	6 (4)	5 (4)	19** (10)	7 (5)	7 (7)
sternebra misshapen						

- No noteworthy findings; * $p<0.05$, ** $P < 0.01$

Toxicokinetics: Plasma exposures (Cmax or AUC) to the individual drug components increased with increase in dose but were not dose proportional. The exposure to amlodipine was lower (50% lower AUC) when amlodipine besylate was administered in combination with valsartan than when administered alone. Amlodipine had no effect on valsartan TK parameters. On gestation day 17, maximum plasma concentration (Cmax) for valsartan was reached between 1 and 2 hr (Table 3.2.1.4), while Cmax for amlodipine was reached between 2 and 8 hr (Table 3.2.1.5).

TABLE 3.2.1.4
EMBRYO-FETAL TOXICITY STUDY IN RATS
TOXICOKINETIC PARAMETERS OF VALSARTAN IN MATERNAL PLASMA

Dose (Val./aml.) [mg/kg/day]	80:5	160:10	320:20	320:0
tmax [h]	1	1	2	1
Cmax [μg/mL]	8.61	14.2	17.9	18.1
Cmax/Dose [μg/mL]/[mg/kg/day]	0.108	0.0888	0.0559	0.0566
AUC(0-24h) [μg*h/mL]	42.1	113	253	250
AUC(0-24h)/Dose [μg*h/mL]/[mg/kg/day]	0.526	0.708	0.790	0.782

TABLE 3.2.1.5
EMBRYO-FETAL TOXICITY STUDY IN RATS
TOXICOKINETIC PARAMETERS OF AMLODIPINE IN MATERNAL PLASMA

Dose (Val./aml.) [mg/kg/day]	80:5	160:10	320:20	0:20
tmax [h]	2	8	8	2
Cmax [ng/mL]	36.4	73.9	131	305
Cmax/Dose [ng/mL]/[mg/kg/day]	7.28	7.39	6.55	15.25
AUC(0-24h) [ng*h/mL]	595.82	1302.60	2889.90	5801.00
AUC(0-24h)/Dose [ng*h/mL]/[mg/kg/day]	119.16	130.26	144.50	290.05

4.0. OVERALL SUMMARY AND EVALUATION

VAA489 (Exforge®) is a fixed dose combination of valsartan and amlodipine besylate. Valsartan is a non-peptidic, orally effective, specific antagonist of angiotensin II, active at the AT-1 receptor. It was developed by Novartis and was approved in 1996 for the treatment of essential hypertension (Diovan®, NDA 20,665). Racemic amlodipine is a dihydropyridine calcium channel antagonist. It was developed by Pfizer and was approved in 1992 as the besylate salt for the treatment of hypertension, chronic stable angina and vasospastic angina (Norvasc®, NDA 19,787). A combination of these drugs is expected to result in an additive or synergistic antihypertensive effect when compared to single drug treatment. Nonclinical studies performed with valsartan:amlodipine combinations include 13 week toxicity studies in rats and marmosets and an embryo-fetal development study in rats. In these studies, the drugs were administered in a ratio of 16:1 (valsartan:amlodipine) on a weight basis.

Repeat Dose Toxicity

Valsartan/amlodipine combinations were administered orally by gavage to rats and marmosets for up to 13 weeks.

Rats

The oral administration of valsartan:amlodipine to Sprague-Dawley rats at doses of 240:15 mg/kg/day for 13 weeks resulted in sacrifice of a male and a female in moribund condition on days 38 and 24, respectively. Gastric irritation (inflammation, ulceration/erosion) was noted for both sexes at doses of 120:7.5 or more mg/kg/day (in males also at 48:3 mg/kg/day) and with valsartan alone (240 mg/kg/day). A follow-up 13 week study in male rats demonstrated no ulceration/erosion at doses lower than 48:3 mg/kg/day. This effect was not observed in the recovery group animals. Kidney findings representing the early stages of chronic progressive nephropathy (non-reversible) were noted in all dose groups. In addition, kidneys from males and females receiving 120:7.5, 240:15 or 240:0 mg valsartan:amlodipine/kg/day exhibited a non-reversible arteriolar medial hypertrophy. This is considered by the sponsor to be a compensatory response to the blood pressure lowering effect of valsartan. Biochemically, moderate increases in BUN, potassium and creatinine were noted in these groups, reflecting a decrease in glomerular filtration rate. Additional histopathological findings included reduced bone marrow erythropoiesis (not noted in recovery group animals) in both sexes treated with the high dose combination or with valsartan alone, and hypertrophy of the adrenal zona glomerulosa (noted with decreased incidence in recovery group animals) in the amlodipine only group. Heart weights (absolute and relative) were nondose-dependently lower (13 to 25%) than control for both sexes in all combination groups and the valsartan alone group, including recovery animals in these groups. This effect was also considered secondary to the expected hypotensive effect of the drug(s) resulting in decreased cardiac afterload. Additional findings included a nondose-dependent decrease (5.5 to 16%) in mean body weight relative to concurrent control at all dose levels. Decreases in erythroid parameters relative to control were noted for both sexes at doses ≥120:7.5 mg (valsartan:amlodipine)/kg/day and 240 mg valsartan/kg/day. The NOAEL was determined to be 16:1 mg (valsartan:amlodipine)/kg/day

Marmosets

Oral administration of valsartan and amlodipine besylate produced severe clinical signs (inappetence, fecal changes (diarrhea, blood and mucoid feces), cold to touch, decreased locomotor activity), reduced food consumption and body weight loss. Two females (one at 160:10 mg/kg/day and another at 160 mg valsartan/kg/day) were sacrificed in moribund condition on study day 8 and found to have erosive/ulcerative inflammation of the cecum and colon. Test substance-related clinical signs (diarrhea, feces with blood and mucus) were noted in animals at 160:10→80:5 mg/kg/day. Body weight loss relative to control or baseline was noted at all dose levels as early as day 10 of the study. Low dose combination animals exhibited weight loss of 6-9% for males and up to 11% for females over the course of the study. High dose combination (160:10 mg/kg/day) animals displayed severe and rapid body weight loss (5-15%) following the first 9 days of treatment. Due to severe body weight loss and clinical signs, dosing for the high dose combination group was suspended from study days 10 to 14 and resumed on study day 15 after decreasing the dose from 160:10 to 80:5 mg/kg/day. A similar change was made to valsartan and amlodipine alone groups with doses reduced to 80 and 5 mg/kg/day, respectively. The valsartan and amlodipine groups also displayed body weight losses with the amlodipine treated males exhibiting the most severe loss (up to 17% by the end of the study). Reduced food consumption was noted at doses ≥80:5 mg/kg/day. A slight decrease in erythroid parameters and a moderate increase in BUN were noted for both sexes at doses of 160:10→80:5 mg/kg/day. Test article-related microscopic pathology was identified in the kidney (exacerbation of a multifaceted nephropathy and medial hypertrophy of renal cortical arterioles) of two females (one each at 160:10→80:5 mg/kg/day and 80 mg valsartan/kg/day). None of these findings were noted in recovery group animals. Moderate to marked erosive or ulcerative inflammation of the intestine (cecum and/or colon) was noted in all drug-treated groups, including recovery animals. The lesions were considered to be associated with amlodipine. Lymphocytic inflammation of the atrial myocardium was noted in individual animals of all drug-treated groups, including one amlodipine dosed recovery male. Test substance-related increased multifocal adrenal cortical vacuolation within the zona fasciculata was present in males treated with the combination or with either valsartan or amlodipine. The findings were absent in recovery group animals. According to the sponsor, this may represent an adaptive response to treatment. A NOAEL was not determined for this study.

Reproductive Toxicity

The valsartan/amlodipine besylate combination was evaluated in rats at oral doses of up to 320:20 mg (valsartan:amlodipine)/kg/day for its effects on embryo-fetal development during organogenesis (days 6 to 17 of gestation). No evidence of teratogenicity was noted. However, maternal toxicity (significant decreases in body weight gain and food consumption relative to control) was noted at 160:10 or more mg/kg/day and at 20 mg amlodipine/kg/day. Valsartan alone did not have any adverse effects on the dams. Maternal toxicity may have caused embryo-fetal toxicity: decreased fetal weight (5%), increased incidence of dilated ureters ($p > 0.05$ on litter basis) and skeletal findings ($p > 0.05$ on litter basis) of misshapen sternebrae and un-ossified forepaw phalanges at 320:20 mg/kg/day. Dilated ureters were also noted at 320 mg valsartan/kg/day ($p < 0.05$ on litter and fetal basis). The NOAELs for maternal and fetal effects were, respectively, 80:5 and 160:10 mg (valsartan:amlodipine) /kg/day.

Toxicokinetics

Comparative systemic exposures at the no observed adverse effect levels (NOAELs) or higher levels of VAA489 in the toxicity species are given in Table 4.1 for valsartan and in Table 4.2 for amlodipine. A NOAEL was not identified in marmosets. The NOAEL exposures in rats were generally similar (0.6 to 2.0 times based on AUC values) to exposures in humans at 320:10 mg (valsartan:amlodipine)/day, the maximum recommended human dose (MRHD). The NOAEL exposures in marmosets were less than a third of the valsartan or amlodipine exposures in humans at the MRHD, indicating the absence of a safety margin for humans.

TABLE 4.1
VAA489: VALSARTAN EXPOSURE MULTIPLES IN TOXICITY STUDIES

Species	Study number/ Rev section	NOAEL[a] (mg/kg)	Gender	AUC_{0-24h} (μg·h/ml)	C_{max} (μg/ml)	Exposure multiples based on	
						AUC_{0-24h}	C_{max}
13-wk rat	0470164	<48:3 [b]	male	40 [d]	13 [d]	0.9	2.3
	3.1.1	<48:3 [b]	female	88 [d]	67 [d]	2.1	11.8
13-wk rat	0570345 3.1.2	16:1	male	17 [c]	3.8 [c]	0.9	2.3
13-wk marmoset	0570032	<40:2.5 [b]	male	44.4 [e]	8.6 [e]	1.1	1.5
	3.1.3	<40:2.5 [b]	female	11.0 [e]	2.6 [e]	0.3	0.4
Embryo-fetal rat	0470007	80:5 dam	female	42 [f]	8.6 [f]	1.0	1.5
	3.2.1	160:10 fetus		113 [f]	14.2 [f]	2.7	2.5

Valsartan exposure multiples are based on the human $AUC_{0-\infty}$ = 41.68 μg·h/ml and C_{max} = 5.72 μg/ml after a single oral dose of 320:5 (valsartan:amlodipine) mg to male and female healthy subjects (Study #VAA489A2310)

TABLE 4.2
VAA489: AMLODIPINE EXPOSURE MULTIPLES IN TOXICITY STUDIES

Species	Study number	NOAEL[a] (mg/kg)	Gender	AUC_{0-24h} (ng·h/ml)	C_{max} (ng/ml)	Exposure multiples based on	
						AUC_{0-24h}	C_{max}
13-wk rat	0470164	<48:3 [b]	male	791 [c]	58.9 [c]	2.6	10.7
	3.1.1	<48:3 [b]	female	1170 [c]	179 [c]	3.8	32.5
13-wk rat	0570345 3.1.2	16:1	male	180 [d]	15.4 [d]	0.6	2.8
13-wk marmoset	0570032	<40:2.5 [b]	male	271 [e]	19.5 [e]	0.9	3.5
		<40:2.5 [b]	female	83 [e]	23.6 [e]	0.3	4.3
Embryo-fetal rat	0470007	80:5 dam	female	596 [f]	36.4 [f]	2.0	6.6
	3.2.1	160:10 fetus		1303 [f]	73.9 [f]	4.3	13.4

Amlodipine exposure multiples are based on the human $AUC_{0-\infty}$ = 306 ng·h/ml and C_{max} = 5.5 ng/ml after a single oral dose of 160:10 (valsartan:amlodipine) mg to male and female healthy subjects (Study #VAA489A2309)
a: No-Observed-Adverse-Effect-Level
b: Lowest dose in study; NOAEL was below, not determined
c: week 10; d: day 72/73; e: day 69/70; f: day 17

Evaluation

VAA489 (Exforge®) is a combination tablet of valsartan (Diovan®, Novartis) and amlodipine besylate (Norvasc®, Pfizer) proposed for the treatment of hypertension. Both drugs have been extensively studied and are widely used as monotherapies for the treatment of hypertension. Their combination is expected to bring an additive or synergistic antihypertensive effect when compared to single drug treatment.

In preclinical toxicity studies, valsartan/amlodipine besylate combinations were associated with changes in the kidney, gastrointestinal tract, erythrocyte parameters, heart and adrenals and each of these effects could be attributed to known effects of one or both components. Of these effects, nephropathy, medial hypertrophy of renal cortical arterioles, decreases in erythroid parameters and decreases in heart weight were associated with valsartan administration. Increases in ulcers in the nonglandular stomach in rats and large intestine (colon and/cecum) in marmosets, inflammation in the right atrium of the heart and adrenal cortical (zona glomerulosa) hypertrophy in marmosets were associated with amlodipine administration. Erosions/ulcers in the glandular stomach (in rats) and large intestine (in marmosets) and adrenal cortical (zona fasciculata) vacuolation (in marmosets) were associated with valsartan and/or amlodipine administration. All of these effects seem to be greater with the combined administration than with valsartan or amlodipine alone. But the sponsor contends that they should not be of major concern because these drugs have been used safely in patients for at least a decade as monotherapies as well as in free combinations. Furthermore, the sponsor argues that the gastrointestinal effects observed in clinical trials with Exforge® were no more frequent with the combination than with the respective monotherapies. VAA489 did not show any teratogenic potential in an embryo-fetal development study in rats. Valsartan:amlodipine at 160:10 mg/kg/day and amlodipine at 20 mg/kg/day were associated with maternal toxicity but had no effects on the developing embryo-fetus. At 320:20 mg/kg/day valsartan:amlodipine and 320 mg/kg/day valsartan, there was an increased incidence of dilated ureters. At 320:20 mg (valsartan:amlodipine)/kg/day, fetal skeletal findings of misshapen sternebrae and un-ossified forepaw phalanges (the latter suggestive of a developmental delay) were noted, which were attributed by the sponsor to significant maternal toxicity. The skeletal findings were regarded as 'variations' and were reported earlier with valsartan (NDA #20,665). Though there were no malformations associated with exposure to the combination in rats, the administration of valsartan (a component in Exforge®) during the 2nd or 3rd trimester of pregnancy is known to be associated with fetal malformations and neonatal deaths. A recent publication notes a significant increase in the risk of cardiovascular and central nervous system congenital malformations with the use of ACE inhibitors during the first trimester.[1] Thus, Exforge® is not recommended for use during pregnancy.

The exposures of animals to valsartan and amlodipine were compared to exposures in humans treated with the highest combination doses of valsartan (320 mg) and amlodipine (10 mg). Exposures to valsartan and amlodipine at NOAEL doses in rats and marmosets were lower or marginally higher than exposures in humans (less than 0.3 times for marmosets and 0.6 to 2.0 times for rats, based on AUC values).

[1] Cooper, W.O. *et al.*: Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med* **354:** 2443-51, 2006
Friedman, J.M.: ACE Inhibitors and Congenital Anomalies. *N Engl J Med* **354:** 2498-00, 2006.

In conclusion, a 16:1 combination of valsartan and amlodipine administered to rats and marmosets had greater adverse effects than treatment with valsartan or amlodipine alone. In spite of this enhancement of toxicity and in spite of the failure to demonstrate a NOAEL for erosive/ulcerative inflammation of the cecum and colon in marmosets, the combination product can still be used safely in humans for the treatment of hypertension as the target organ toxicities are monitorable and attributable to the effects of the individual components of the combination, which have been used, often concomitantly, to treat hypertensive patients since their respective approvals for this indication (1992 for amlodipine besylate and 1996 for valsartan).

Recommendations on Labeling: See page 6

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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/s/

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